Novel Approach to Quantification of Telomere Length With Direct Nanopore Sequencing and PCR Amplification

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Introduction

Space as an alien environment imposes a range of foreign stresses on the human body. Astronauts are exposed to microgravity, radiation and other external and internal stressors, all of which have significant and sometimes detrimental effects on health (Figure 1).

From the loss of critical density on the bone, to the weakening of the cardiovascular system, almost all critical systems of the body are affected. Through many years of research, NASA has been able to identify several mechanisms of space-induced changes, and develop ways for astronauts to combat these effects on the body when in space.

Figure 1: Effects of Space Flight on the Human Body

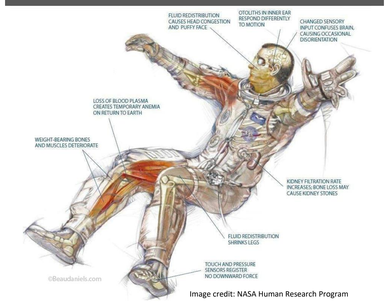


Image credit: NASA Human Research Program

However, while physical effects on the body are visible or scanable, the molecular changes in the body are not apparent. A mechanism of molecular measurement of general cellular health in space would enable more in-depth research into how space related stressors effect organisms, and could also be developed into a metric to indicate physical health status of astronauts on longer flight missions.

Background

Novel approach to telomere length measurement

Minimum time and effort.

Single base pair resolution.

Real-time measurement on flight missions.

Overview of Telomeres

- Tandem array of repeating DNA sequences that serve as protection mechanisms for chromosomal ends.
- Shorten in length with every cell division, at a certain point, the cell can no longer divide.
- An important factor for cellular vitality and an indicator for overall physical wellness.

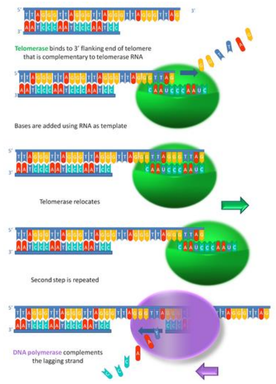


Figure 2: Telomere elongation during cell cycle proliferation

Twin Telomere Study

- Year-long twin study showed lengthening of telomeres in space.
- May have been an induced-response to stresses in space.
- Data could only be obtained after twin astronaut return to Earth.

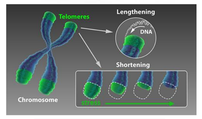
A New Methodology

- Previous methods only provide estimation of telomere length across all 23 chromosomes.
- Oxford Nanopore MinION: a portable compact sequencing device suitable for long reads of genomic DNA.

Project Goals

- **To develop a telomere length measurement technique utilizing MinION sequencing technology with single base pair resolution for fundamental molecular research.**
- **To utilize this technology for assessing the effects of environmental stresses in space on telomere length in a model cell line experiments.**
- **Develop from basic science research a general health monitoring metric utilizing telomere length monitoring in real time with no need for communication with ground.**

Image credit: Oxford Nanopore

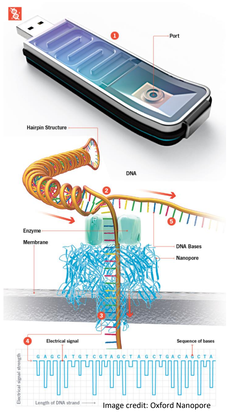


Preliminary Experiment Design

A)

Image credit: Clarmont BioSolutions

B)



C)

Image credit: Oxford Nanopore

Figure 3: Method Summary A) gDNA isolation; B) MinION sequencing; C) Word find and count of telomeric repeats.

1. Genomic DNA was isolated from B6129SF1/J wild type female bone marrow flushed using Claremont BioSolutions SimplePrep X8 Instrument.
2. Genomic library Preparation used the Oxford Nanopore 1D^2 Sequencing Kit.
3. DNA was sequenced on the Oxford Nanopore MinION. Sequence data was basecalled using the ONT-Albacore program and converted to FASTA format via the poRe opensource software for analysis.
4. Previously identified pretelomere sequences from *mus musculus* chromosome 4 were aligned with sequence data using NCBI BLAST. Then the position of these pretelomere sequences visualized using NCBI Genome Decoration relative to the telomeric repeats "TTAGGG".
5. The number of telomere tandem repeats of "TTAGGG" that followed the pretelomere sequences were counted with Microsoft Word Count tool.

Sequencing Data

Figure 4: MinKNOWN local basecalling real time presentation of read length and quality score.

In situ basecalling using the Oxford Nanopore MinKNOWN software illustrates high quality scores for long read sequences (>6000 bp)

Mapping PreTelomere Sequences

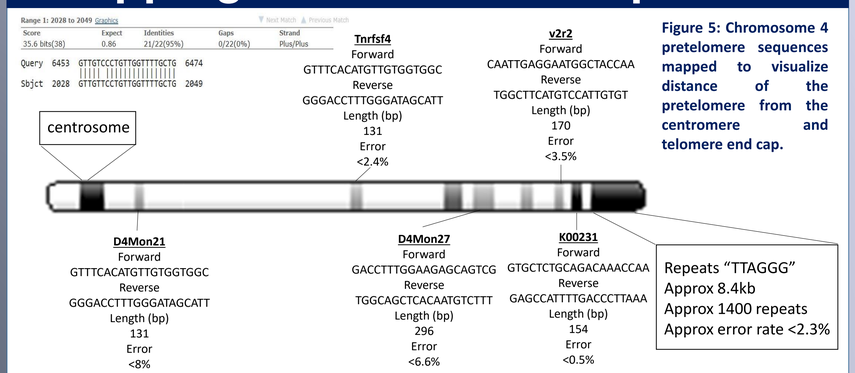


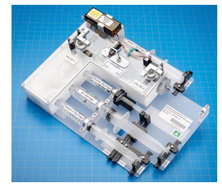
Figure 5: Chromosome 4 pretelomere sequences mapped to visualize distance of the pretelomere from the centromere and telomere end cap.

Conclusions

- **Telomere length has been successfully obtained at single chromosome, single base pair resolution utilizing the handheld MinION sequencing technology and common open source data processing programs.**
- **Additionally, pretelomere sequences from chromosome 4 were mapped and primer/probes designed to amplify the telomere region only.**

Future Methods Design

1. During a flight experiment gDNA would be isolated using the **WetLab-2 Sample Preparation Module (SPM)** which has been flight validated on station.



2. After gDNA isolation pre-telomeric primers, identified in preliminary studies, would initiate **PCR expansion to amplify the telomeric region**.
3. **Ligation of barcodes** enable multi-sample reads providing a platform for conducting **statistically significant research** in a single run of the sequencer.
4. MinION **sequencing run** and **local basecalling** requiring no internet connection or cloud based software.
5. Post sequencing run analysis of **telomere tandem repeats** using an automated Command Prompt script to auto populate tabulated results for user friendly assay.

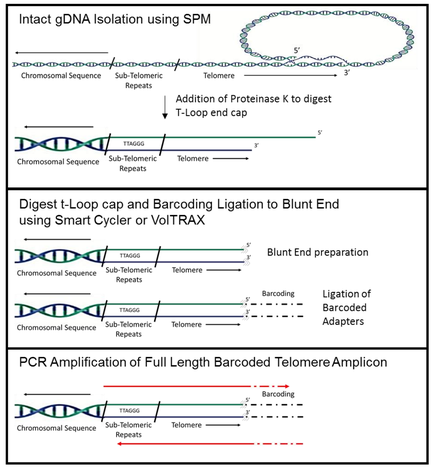


Figure 6: Theoretical amplification of the telomere region and barcoding methodology.

This experimental regime is optimized for application in flight; minimizing astronaut time and hands-on interaction with the experiment.